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A modified fluorescent microsphere-based approach for determining resting and hyperemic blood flows in individual murine skeletal muscles

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Abstract

The goal of this study was to develop a modified fluorescent microsphere-based approach for measuring resting and hyperemic blood flows in individual mouse skeletal muscles. Absolute resting blood flow in the left gracilis posterior was 1.04±0.12 ml·min^{-1}·g^{-1}, while functional hyperemia following muscle activity was 5.94±1.33 ml·min^{-1}·g^{-1}. Measuring absolute blood flow requires sampling arterial blood that serves as a flow-rate and concentration reference to the fluorescent microsphere (FMS) content in the tissue-of-interest for calculating the flow value. Because sampling arterial blood can impair cardiovascular function in the mouse, we also modified our FMS approach to determine relative blood flows in the left gracilis posterior by using the contralateral muscle as our reference in blood flow calculations. Absolute and relative hyperemia measurements detect similar increases in blood flow — 521.93±216.76% and 555.24±213.82%, respectively. However, sampling arterial blood during absolute blood flow measurements significantly decreased mean arterial pressure from the beginning to the end of our experiments, from 102.7±2.18 to 75.5±9.71 mm Hg. This decrease was not seen when measuring relative blood flows. This approach provides critical advantages over contemporary blood flow measurement approaches by allowing blood flow measurements in small and non-superficial tissues.

Keywords: Blood flow; Mouse; Fluorescent microspheres; Skeletal muscle; Hyperemia

1. Introduction

The capacity to match blood flow to tissue need is essential for normal functioning in any tissue. For skeletal muscle, increasing its blood supply through functional hyperemia is essential during locomotion or physical activity. During ischemic revascularization, functional hyperemia in skeletal muscle vasculature is impaired (Hudlicka et al., 1994). Similarly, patients with peripheral artery disease experience intermittent claudication, or ischemic pain, with locomotion (Lumsden and Rice, 2006) that is indicative of an impaired functional hyperemia (Arosio et al., 2002). Additionally, peripheral artery disease is associated with increased mortality due to coronary artery disease (Aronow, 2005). Thus, a deficit in the capacity for functional hyperemia will impair normal tissue function and is suggestive of underlying cardiovascular complications.

Our laboratory utilizes a murine model of chronic hindlimb ischemia (Sullivan et al., 2002) to examine the mechanisms controlling ischemic revascularization. Specifically, we utilize the model to investigate the impairment of functional capacity in newly formed or repaired vasculature following chronic ischemia (Hudlicka et al., 1994).

An important aspect of the mouse model of hindlimb ischemia is that revascularization is spatially segregated; microvascular growth (angiogenesis and arteriolarization) occurs predominately in the lower leg and large vessel remodeling (collateralization) occurs predominately in the upper leg (Sullivan et al., 2002). Because feed arteries and arterioles may utilize different molecular pathways to restore proper

Abbreviations: FMS, fluorescent microspheres; LCCA, left common carotid artery; RCCA, right common carotid artery; RFA, right femoral artery; MAP, mean arterial pressure.

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vascular reactivity following ischemia, it would be beneficial to assay blood flow in these different regions.

Unfortunately, due to the limited size of the mouse, contemporary methods for determining blood flow lack the resolution to determine blood flow to individual skeletal muscles and/or are limited to the most superficial tissues. For example, MRI is limited to global measures of hindlimb blood flow (Helisch et al., 2006), while ultrasound-based blood flow measurements are limited to large caliber vessels such as the carotid (Sullivan and Hoying, 2002) or femoral (Williams et al., 2006) arteries. At the other end of the spectrum, live tissue imaging with intravital microscopy, which provides the highest level of tissue resolution (Bearden et al., 2004; Duza and Sarelius, 2004), is generally limited to measures of arteriolar reactivity rather than blood flow, due to the requirement that vessel segments must reside in a single plane to obtain sound velocity measurements. Additionally, intra-vital approaches are usually limited to sheet-like tissue, such as the cremaster muscle, due to the challenge of resolving arterioles in optically dense skeletal muscle. The most commonly utilized blood flow measurement technology, laser Doppler perfusion imaging (LDPI), which determines perfusion based on red cell velocity and hematocrit, can be resolved to the single tissue level by post hoc determinations of regions-of-interest from LDPI scans (Chalothorn et al., 2005; Sullivan et al., 2002). However, even when utilizing deep-penetrating probes (Chalothorn et al., 2005; Yu et al., 2005), LDPI is still limited to assessing perfusion in the most superficial tissues.

Another approach for measuring blood flow is with fluorescent microspheres (FMS). For this approach, blood flow is calculated by comparing the tissue deposition of intra-arterially injected FMS and the FMS content of arterial blood that was sampled at a fixed withdrawal rate. Thus, FMS-based approaches can be used to measure blood flow in any tissue receiving arterial blood if the FMS are uniformly distributed in that blood. However, traditional FMS-based approaches have limited tissue resolution in mice. Statistical models indicate that 200–400 FMS must lodge in a tissue to accurately measure blood flow (Prinzen and Bassingthwaighte, 2000) when using fluorimetry, the most commonly used tool for determining tissue fluorescence. This usually requires harvesting most or all hindlimb skeletal muscle to obtain enough FMS (Kubis et al., 2002). Additionally, FMS have not been used to measure resting blood flow and hyperemia in a single mouse, except when alternative referencing approaches are used (Maxwell et al., 1998). This is probably due to the fact that multiple arterial withdrawals have not been successfully taken from mice (Richer et al., 2000), likely due to their low blood volume.

In summary, none of the currently available methods for blood flow measurement allow us to resolve resting blood flow and functional hyperemia in individual skeletal muscles throughout the murine hindlimb. However, FMS present the best alternative in that they can be used to measure blood flow in all skeletal muscles, superficial or deep. Therefore, the goal of this project was to develop a modified fluorescent microsphere-based approach that was capable of measuring resting blood flow and functional hyperemia in individual muscles of the same mouse.

2. Methods

2.1. Animals

Male and female FVB/N transgenic mice, between 2 and 6 months of age, that express green fluorescent protein (GFP) in vascular endothelium (Motoike et al., 2000) were used for all experiments according protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Four mice were used to determine absolute blood flow, while five mice were used to determine relative blood flow. Mice were obtained from a breeding colony maintained at the University of Arizona on a 12:12 light:dark cycle and given water and rodent chow ad libitum.

2.2. Surgical Instruments

S&T forceps (angled 45°), Dumont forceps (#5–45), Castroviejo needle holder, Metzenbaum scissors, Iris scissors (curved, 11.5 cm), S&T clamp applying forceps, and S&T vascular clamp (B-1) were obtained from Fine Science Tools (Foster City, CA, USA).

2.3. Anesthesis and animal preparation

All mice were initially anesthetized in an anesthesia box with 5% isoflurane (Abbott, Abbott Park, Illinois, USA) administered through an isoflurane vaporizer and balanced with oxygen flowing at ~3.5 $l \cdot min^{-1}$ (VT-110 and VT525, JD Medical, Phoenix, AZ, USA). Following induction, anesthesia was maintained throughout animal preparation and experimentation with 1–2% isoflurane balanced with oxygen flowing at 0.5–1.0 $l\ min^{-1}$ through a small animal anesthesia mask (Harvard Apparatus, Holliston, MA, USA). The mask was modified by removing the mask portion and repositioning the rubber diaphragm over the fitting port to improve access to the ventral surface of the neck during carotid artery catheterizations (see below). Prior to experimentation, hair on the ventral neck and hindlimbs was removed with trimming clippers and depilatory cream (Nair, Princeton, NJ, USA). During experiments, body temperature was maintained with a water-circulating thermal pad (Gaymar, Orchard Park, NY, USA). Additionally, we found it important to cover the animal with two to three gauze sponges or a small towel to prevent ventral heat loss which likely causes hypothermic vasoconstriction and other cardiovascular complications.

2.4. Arterial catheterization

Following exposure from its neurovascular bundle, the right common carotid artery (RCCA) was catheterized with a polyethylene 50 (427411, Thomas Scientific, Swedesboro, NJ, USA) catheter that was tapered over heat to the approximate diameter of the vessel, ~0.5 mm (Fig. 1). The catheter was placed through a small incision made 1/2-way through the artery at ~45° to the longitudinal axis. Temporary placement of a vascular clamp downstream to the catheterization site and ligation of the RCCA